Correlation of the antioxidant level in saliva and periodontal status in Ukrainian children with atopy

Kryvenko Liudmyla

Abstract

Objective: To find correlation between the total antioxidant and periodontal status in the saliva of periodontally compromised and not compromised patients, respectively, with atopy, and healthy controls (HC) to assess diagnostic utility of malondialdehyde (MDA), glutathione and superoxide dismutase.

Methods: The study was conducted on 141 subjects, who were in the age group of 12–18 years. Among the 141 subjects, 55 HC formed Group HC. The atopy group consisted of 126 patients: 76 patients of atopic diseases (AD) and gingivitis (Group ADG) and 50 patients of AD without gingivitis (Group AD). The level of gingival inflammation was evaluated using the sulcus bleeding index (SBI) reported by Mühlemann and Son. Glutathione, MDA and superoxide dismutase were estimated in saliva. Data were statistically analyzed by Microsoft Excel using unpaired t-test for significance of differences between each group, and regression analysis was done.

Results: The mean salivary MDA levels of group HC was 3.81 ± 0.83 µm/L. The mean salivary levels of group ADG was 6.87 ± 0.91 µm/L and group AD was 5.96 ± 0.79 µm/L. The mean salivary MDA levels of group HC was significantly lower compared to group ADG and AD (P < 0.05). Similarly, in the case of salivary glutathione, marker levels revealed a significant decrease (P < 0.05) when mean values in the control group (5.12 ± 0.66 µmol/L) were compared with the ADG group (2.31 ± 0.44 µmol/L) and AD groups (2.69 ± 0.56 µmol/L), but the change was not significant (P > 0.05) when AD and ADG patients were compared. The SOD activity was significantly lower in the ADG and AD groups compared to healthy children.

Conclusions: The results showed changes in antioxidant balance in children with atopy. The regression analysis showed the absence of correlation between the level of antioxidants and inflammation in periodontal tissues.

Keywords: gingivitis, antioxidants, atopy

Introduction

Prevalence of atopic diseases (AD) such as bronchial asthma, allergic rhinitis, atopic dermatitis, has been progressively increasing in children all over the world. The International Study of Asthma and Allergies in Childhood (ISAAC) has mapped out a significant variation and increase in the prevalence of childhood allergies across many countries1–3.

On the other hand, periodontal problems are often observed in child age. Lipid peroxidation (LPO) has been implicated in the pathogenesis of several pathologic disorders, including periodontal disease. Studies investigating the use of saliva as a diagnostic fluid have a long history.

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This noninvasive approach is not limited only to the diagnosis of oral diseases, because many systemic diseases, such as different types of cancers, cardiovascular diseases, immunologic syndromes, and hereditary deficiencies, can also be studied with the aid of salivary diagnostics4–6.

Saliva is an accessible biofluid that contains components derived from the mucosal surfaces, gingival crevices, and tooth surfaces of the mouth. Saliva also contains microorganisms that colonize the mouth and other exogenous substances and so can potentially provide an insight into the relationship of the host with the environment7.

Currently, there is growing interest in the linkage between antioxidants and periodontal disease. Antioxidants include superoxide dismutase (SOD), uric acid, ascorbic acid, α-tocopherol, glutathione and albumin. One important antioxidant, SOD catalyzes the dismutation of the superoxide
Superoxide dismutase has also been localized within the human periodontal ligament and may represent an important defense mechanism within gingival cells against superoxide release\(^1\). Malondialdehyde (MDA) is one of many low molecular weight end products of LPO\(^2\). As MDA is a highly toxic aldehyde molecule, it is considered to be an ideal marker of LPO\(^3\).

This study aimed to find correlation between the total antioxidant and periodontal status in the saliva of periodontally compromised and not compromised patients with atopy, and healthy controls to assess diagnostic utility of MDA, glutathione and superoxide dismutase.

**Materials and Methods**

A cross-sectional study was conducted on 141 subjects, which were in the age group of 12–18 years reporting to the Department of Pediatric Dentistry. Among the 141 subjects, 55 healthy controls (HC) formed (Group HC). The atopy group consisted of 126 patients with the following conditions: 76 patients of AD and gingivitis (Group ADG) and 50 patients of AD without clinical manifestations of gingivitis (Group AD). Patients with systemic illness except bronchial asthma, atopic dermatitis, allergic rhinitis, previous history of malignancy or history of antioxidant medication were excluded. Ethical approval was obtained from the Ethical Committee of Kharkiv National Medical University and informed consent was obtained from all the study subjects (their parents). Unstimulated saliva was collected from the study subjects between 9:00 am and 12:00 pm to avoid diurnal variation. The subjects were requested not to eat, drink, perform oral hygiene activities or chew 60 min prior to the saliva collection procedure. The subjects were then seated on the dental chair and asked to spit in a graduated container every 1 min till 5 ml of saliva was obtained. During saliva collection, subjects were instructed not to speak or swallow. The salivary samples were stored at a temperature of −20°C. MDA. The marker of LPO was estimated as thiobarbituric acid-reactive substances (TBARS)\(^4\). To 1 mL of sample, 1.5 mL of 0.8% thiobarbituric acid (TBA) was added. Then, 1.5 mL of acetic acid and 0.4 mL of 8.1% sodium dodecyl sulfate were added. Distilled water was added to make the mixture up to 5 mL, and it was then placed in a hot water bath at 95°C for 1 h. The mixture was allowed to cool, and 5 mL of pyridine and n-butanol (15:1, v/v) along with 1.0 mL of distilled water were added. The mixture was vortexed and centrifuged at 4,000 rpm for 10 min. With a spectrophotometer, absorbance of the upper layer was measured at 532 nm against distilled water. When allowed to react with TBA, MDA formed a colored complex that was measured using the spectrophotometer.

Glutathione was estimated using the method of Beutler et al. based on reduction of 5,5’-dithiobis-(2 nitrobenzoic acid) (DTNB) by GSH. The technique employs meta-phosphoric acid for protein precipitation, and the supernatant obtained on reaction with 5-5’dithiobis2-nitrobenzoic acid resulted in a yellow-colored derivative that was assayed with a spectrophotometer at 412 nm\(^5\).

Superoxide dismutase activity was analyzed by the reduction of nitroblue tetrazolium (NBTr) by superoxide, which formed formazan and detected spectrometrically at 560 nm using Genesys 10 UV and expressed in terms of U/mL\(^6\).

The level of gingival inflammation was evaluated using the SBI reported by Mühlemann and Son. The SBI was recorded on six tooth surfaces (mesio-buccal, buccal, disto-buccal, mesio-longual/palatal, lingual/palatal, and disto-lingual/palatal). The scores for the SBI were: 0: no bleeding, 1: bleeding on probing with no change in color and no swelling, 2: bleeding on probing with a change in color and no swelling or macroscopic edema, 3: bleeding on probing with a change in color edematous swelling, 4: bleeding on probing with a change in color due to inflammation, edematous swelling with ulceration, 5: spontaneous bleeding, changes in color and marked swelling with ulceration.

Statistical analysis of the data was performed by using the Microsoft Office software. Data were statistically analyzed by Microsoft Excel using unpaired t-test for significance of differences between each group and regression analysis by least squares method, which is a statistical process for estimating the relationships among variables. \(P\) values of less than 0.05 were considered to be statistically significant. The normality of data was checked before the statistical analysis was performed.

**Results**

The mean salivary MDA levels of group HC was 3.81 ± 0.83 µmol/L. The mean salivary levels of group ADG was 6.87 ± 0.91 µmol/L and group AD was 5.96 ± 0.79 µmol/L.

The mean salivary MDA levels of group HC was significantly lower compared to group ADG and AD (\(P < 0.05\)). The mean salivary MDA levels of group ADG and group AD did not have significant difference (Table 1).

Similarly, in the case of salivary glutathione, marker levels revealed a significant decrease (\(P < 0.05\)) when mean values in the control group (5.12 ± 0.66 µmol/L) were compared with the ADG group (2.31 ± 0.44 µmol/L) and AD groups (2.69 ± 0.56 µmol/L) (Table 1), but the change was not significant (\(P > 0.05\)) when AD and ADG patients were compared (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Evaluation and comparison of mean ± SD values for salivary MDA and GSH in controls, ADG, AD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>MDA µmol/L</td>
</tr>
<tr>
<td>ADG</td>
<td>6.87 ± 0.91</td>
</tr>
<tr>
<td>AD</td>
<td>5.96 ± 0.79</td>
</tr>
<tr>
<td>HC</td>
<td>3.81 ± 0.83</td>
</tr>
</tbody>
</table>

\(P < 0.05\) significant.
The SOD activity was significantly lower in the ADG and AD groups compared to healthy children. In saliva, the superoxide dismutase concentration was equal to $3.3 \pm 0.41$ U/L in group of atopy and gingivitis children and $3.27 \pm 0.4$ U/L in group of atopy children. There was no significant difference in the level of superoxide dismutase concentration between these two groups.

The mean SBI index of group ADG was $3.42 \pm 0.42$. The mean SBI level in AD group was equal to $0.55 \pm 0.52$. The HC group level was $0.57 \pm 0.6$ (Table 2).

The comparison of mean values of SBI shows significant higher level of inflammation in periodontal tissues in ADG group of patients compared to AD and HC groups.

For estimating the relationships between SBI index and level of MDA in the ADG group, regression analysis was done (Table 3). As $R^2$ was equal to 0.432358, there is very weak correlation between level of malondialdehyde level and periodontal problems.

The regression analysis method demonstrates that regression function cannot be built in the group of ADG patients. The value of $R^2$, generalized $R^2$, multiple $R$ prove that an inflammation in periodontal tissues that is measured by SBI index does not correlate with the level of malondialdehyde (Table 3).

The similar situation is observed, if SBI index (in regression function $Y$) and MDA (in regression function $X$) level is analyzed in AD group. Multiple $R$ was $0.012526$, $R^2$ was $0.000157$, generalized $R^2$ was $–0.02067$.

The regression statistics in AD group shows that there is no correlation between SBI index and malondialdehyde level. In clinical practice, it means that the level of MDA cannot be used for SBI prognosis. Increased level of MDA is not always revealed in patients with clinical signs of gingivitis, but can be observed in preclinical stage of development of gingivitis.

There was no correlation between SBI (in regression function $Y$) and glutathione (in regression function $X$) level in AD and ADG group of patients (Table 4).

In AD group, $R^2$ was equal to $0.031482$, generalized $R^2$ was $0.011304$, multiple $R$ was $0.177431$ which means that there is no correlation between inflammation in periodontal tissues and level of glutathione. In ADG group we observed similar situation. Multiple $R$ was equal to $0.090662$, $R^2$ was $0.00822$, generalized $R^2$ was $–0.00518$.

The results of regression analyses showed there was no correlation between the sulcus bleeding index and the SOD activity (Table 5).

In the ADG group, $R^2$ was equal to $0.106407492$, multiple $R$ was $0.326201613$, generalized $R^2$ was $0.094331918$. Almost similar situation is observed in the AD group: $R^2$ was equal to $0.01162882$, multiple $R$ was $0.107837008$, generalized $R^2$ was $–0.00862246$.

**Discussion**

Regression analysis is widely used for prediction where its use has substantial overlap with the field of machine learning. Regression analysis is also used to understand which among the independent variables are related to the dependent variable, and to explore the forms of these relationships. In restricted circumstances, regression analysis can be used to infer causal relationships between the independent and dependent variables.

Of the many biological targets of oxidative stress, lipids are the most commonly involved class of bio-molecules. Lipid oxidation gives rise to a number of secondary by-products. MDA is the principal and most widely studied product of polyunsaturated fatty acid peroxidation.

| **Table 2** Evaluation and comparison of mean ± SD values for SBI index |
|---|---|
| **Groups** | **SBI** |
| ADG | $3.42 \pm 0.42$ |
| AD | $0.55 \pm 0.52$ |
| HC | $0.57 \pm 0.6$ |

*P* < 0.05 significant.

| **Table 3** Regression analyses of SBI and MDA correlation in AD and ADG group |
|---|---|
| **Regression statistics** | **AD** | **ADG** |
| Multiple $R$ | $0.012526$ | $0.657539$ |
| $R^2$ | $0.000157$ | $0.432358$ |
| Generalized $R^2$ | $–0.02067$ | $0.424687$ |
| Standard deviation | $0.527224$ | $0.317763$ |
| Observations | 50 | 76 |

| **Table 4** Regression analyses of SBI and glutathione correlation in AD and ADG group |
|---|---|
| **Regression statistics** | **AD** | **ADG** |
| Multiple $R$ | $0.177431$ | $0.090662$ |
| $R^2$ | $0.031482$ | $0.00822$ |
| Generalized $R^2$ | $0.011304$ | $–0.00518$ |
| Standard deviation | $0.518899$ | $0.420024$ |
| Observations | 50 | 76 |

| **Table 5** Regression analyses of SBI and SOD correlation in AD and ADG group |
|---|---|
| **Regression statistics** | **AD** | **ADG** |
| Multiple $R$ | $0.107837008$ | $0.326201613$ |
| $R^2$ | $0.01162882$ | $0.106407492$ |
| Generalized $R^2$ | $–0.008962246$ | $0.094331918$ |
| Standard deviation | $0.524190256$ | $0.398690311$ |
| Observations | 50 | 76 |
Its interaction with DNA and proteins has often been referred to as potentially mutagenic and atherogenic.\(^1,8,19\)

Changes in the salivary antioxidant enzymes suggest that saliva may be an appropriate marker for the prognosis of oral diseases compared to the conventional invasive serum antioxidant enzyme.\(^20\)

In this study, a significantly higher salivary MDA level was observed in the AD and ADG groups, when compared to the HC group. This is in accordance with recent studies where scientists found significantly elevated levels of LPO products when compared to controls.\(^21,24\)

Chapple et al.\(^25\) showed that patients with compromised periodontium had a low total antioxidant status. Other group of scientists\(^26\) reported that the level of antioxidants was lower in the peri-implant disease group than healthy controls. There is a study, which proved the absence of difference in the antioxidant level in the saliva between patients with periodontal disease and healthy controls.\(^27\)

This study suggests that salivary MDA and glutathione could serve as a potential diagnostic marker in children with atopy. However, regression analysis showed that there is no correlation between inflammation in periodontal tissues and level of antioxidants. The obtained data prove that there is an oxidative misbalance in children with atopy. Level of MDA is increased and level of glutathione is decreased in group of patients with atopy, and it does not depend on the presence of gingivitis in these groups.

As there was no significant difference in the level of superoxide dismutase concentration between groups of atopy children with gingivitis and health periodontium, we suggest that antioxidant imbalance is primarily explained by atopic disease.

**Conclusion**

The present study has been done to evaluate salivary LPO and periodontal status and to find the correlation between antioxidant level in unstimulated saliva of children and inflammation in periodontal tissues. The results showed changes in antioxidant balance in children with atopy. The regression analysis that was done showed the absence of correlation between level of antioxidants and inflammation in periodontal tissues.

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Nil.

**Conflicts of Interest**

There are no conflicts of interest.

**References**


